Appendix A: Publications which reflect the level of skill in the art regarding the importance of antibody CDR3 domains and the use of these domains to generate other antibodies with the same binding specificity

#	Author	Citation	Comments
1	Berezov *et al.*	(2001) BIAjournal 8(1): Scientific Review 8 [Biacore Journal]	A series of peptide mimetics based on the CDR3 of anti-HER2 mAb (4D5) was created. HER2 is a member of the tyrosine kinase receptor family which includes EGFR and is highly homologous to EGFR. The peptides were found to (1) bind to HER2 with high affinity; (2) compete with the parent Ab (4D5) for binding; and (3) diminish the signaling properties of HER2.
2	Klimka *et al.*	(2000) British J. of Cancer 83(2):252-260	A humanized anti-CD30 antibody using only the heavy chain variable CDR3 domain of a murine anti-CD30 antibody, Ki-4 was produced. The humanized antibody was found to compete with the parental murine antibody for binding and to retain other functional characteristics of the parental murine antibody (*e.g.*, inhibits the shedding of the extracellular part of the CD30 receptor from L540 cells).
3	Beiboer *et al.*	(2000) J. Mol. Biol. 296:833-849	An antibody to epithelial glycoprotein-2 (EGP-2) was engineered by retaining only the murine heavy chain CDR3 domain of the murine MOC-31 antibody. The newly created antibody was found to bind the same epitope and have a similar binding affinity as the parental murine antibody.
4	Rader *et al.*	(1998) PNAS USA 95:8910-8915	Rader *et al.* describe the production of a humanized anti-integrin $\alpha_v\beta_3$ antibody using the heavy and light chain variable CDR3 domains of the murine anti-integrin $\alpha_v\beta_3$ antibody, LM609. Several antibodies were produced having different sequences outside the CDR3 regions and capable of binding the same epitope as the parent murine antibody with affinities as high or higher than the parent murine antibody.

5	Barbas *et al.*	(1994) J. Am. Chem. Soc. 116: 2161-2162	Barbas *et al.* describe a method for generating antibodies having high affinity for double-stranded DNA and successfully generated isolated antibodies by antigen selection from synthetic libraries which utilized the same heavy chain with randomized CDR3 sequences. The authors concluded that the CDR3 provides the most significant contribution to antigen binding (page 2161, left column, second full paragraph).
6	Barbas *et al.*	(1995) PNAS USA 92:2529-2533	Barbas *et al.* describe grafting the heavy chain CDR3 sequences of three Fabs, SI-1, SI-40 and SI-32, against human placental DNA onto the heavy chain of an anti-tetanus toxoid Fab, thereby, replacing the existing heavy chain CDR3. The results of these studies showed that grafted Fabs produced binding to DNA (page 2532, second paragraph, and the Abstract) and, thus, that the CDR3 alone conferred binding specificity.
7	Ditzel *et al.*	(1996) J. of Immunol. 157:739-749	Describe grafting studies which showed that a heavy chain CDR3 domain can be transferred to the heavy chain of another antibody and retain the same binding specificity. Specifically, the heavy chain CDR3 sequence of the polyspecific Fab LNA3 was grafted onto the heavy chain of the monospecific IgG tetanus toxoid-binding Fab p313, thus, replacing the existing heavy chain CDR3 (paragraph bridging columns on page 740). The binding specificity of the LNA3 heavy chain CDR3-grafted Fab (LNA3/p313) was tested in an ELISA against a panel of exogenous and autoantigens (Figure 3). LNA3/p313 bound to the panel of antigens as did the original LNA3 Fab (page 742, second column, through page 744, first column and Figure 3e).
8	Igarashi *et al.*	(1995) J. Biochem. (Tokyo) 117:452-457	Using an antibody which bound phosphatidylserine (PS), Igarashi *et al.* found that a 12-amino acid synthetic peptide corresponding to the CDR3 domain of the heavy chain bound specifically to PS and possessed a similar specificity as the parent antibody, although the affinity of the peptide to PS was lower. The specific binding of the peptide was confirmed by routine techniques, such as ELISA and TLC-immunostaining.

9	Bourgeois *et al.*	(1998) J. Virol. 72:807-810	Showed that a single peptide derived from the heavy chain CDR3 domain of an antibody against respiratory syncytial virus (RSV) was capable of neutralizing the virus *in vitro* and protected mice against RSV lung infection.
10	Levi *et al.*	(1993) PNAS 90:4374-4378	Describe a peptide based on the heavy chain CDR3 domain of a murine anti-HIV, F58. The peptide was found to cross react with parent antibody (F58) and specifically inhibit the parent antibody from binding HIV. The peptide was also shown to possess antiviral activity by inhibiting HIV-1 replication and syncytium formation by infected cells.
11	Polymenis and Stoller	(1994) J. Immunol. 152:5218-5329	Polymenis and Stoller showed that a single chain variable fragment (scFv) not capable of binding a specific antigen, Z-DNA, could be converted into a Z-DNA-binding scFv by grafting the heavy chain CDR3 region of a Z-DNA-binding antibody onto it (Fig. 3). Polymenis and Stoller also showed that significant Z-DNA binding was retained even when the heavy chain CDR3 of a functional Z-DNA binding antibody was combined with other heavy chain variable sequences which only had 44% sequence identity with the corresponding sequences of the variable region from which the heavy chain CDR3 region was derived.
12	Xu and Davis	(2000) Immunity 13:37-45	Xu and Davis report that diversity at the heavy chain CDR3 is sufficient to permit otherwise identical IgM molecules to distinguish between a variety of hapten and protein antigens. "These results are consistent with a model in which the highly diverse CDR3 loops are the key determinant of specificity in antigen recognition in both T cell receptors (TCR) and antibodies, whereas germline-encoded CDR1 and CDR2 sequences are much more cross-reactive."